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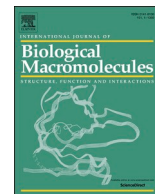
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Animal-free coacervates: The combination of fungal chitosan-gum Arabic for the encapsulation of lipophilic compounds

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ABSTRACT

In this study, fungal chitosan (FC) and gum Arabic (GA) were combined to develop non-animal complex coacervates for encapsulation. Optimal coacervate formation occurred at pH 5 with a 1:4 (FC:GA) weight ratio. Innovative complementary approaches, including rheology coupled with phase-contrast microscopy, revealed that FC-GA coacervates could withstand high shear rates, reverting to their original structure afterward, making them suitable for industrial applications. FTIR, DSC, and TGA analyses confirmed the electrostatic interactions and thermal stability, making them suitable for high-temperature procedures like spray-drying or extrusion. Higher GA concentrations increased coacervate hydrophilicity, while low-dielectric-constant liquids reduced particle size and disrupted coacervates. This study also explored interactions with solvents used in cosmetics, finding that isohexadecane, ethylhexyl stearate, and ethanol improved wetting properties by reducing electrostatic interactions, while polar solvents such as water and glycerol hindered them due to stronger interactions. The coacervates effectively encapsulated α -tocopherol, achieving an 82.6 % of encapsulation efficiency at a 1:1 (w/w) wall material-to-active ratio. These findings highlight the potential of FC-GA coacervates as stable, easy-to-prepare encapsulation materials for high-shear and high-temperature conditions, offering promising applications in the food, cosmetic, and pharmaceutical sectors.

1. Introduction

Microencapsulation is a technique allowing the encapsulation of a core (in liquid, solid, or gas form) in a protective shell (usually made of polymeric materials) that can be permeable, semi-permeable, or completely impermeable, depending on the final application. This technique has found numerous applications in fields such as cosmetics, food industry, pharmaceutical industry, or textiles. Microencapsulation aims to isolate and protect the core material from the external environment, thereby enhancing its stability, extending its shelf life, providing controlled release of the encapsulated substance and thus improving the efficiency of the final product [1].

Complex coacervation is one of the most effective methods of microencapsulation, as it allows high payload efficiency, strong oxidation stability, and versatility in encapsulating a wide range of core materials (both lipophilic and hydrophilic) with a variety of delivery mechanisms [2]. The complex coacervation process involves the electrostatic interaction between two oppositely charged polymers (usually

a protein and a polysaccharide), resulting in the formation of a coacervate phase that encapsulates the core material [3]. One common and oldest combination of polymers used for this technique is gum Arabic (GA) and gelatin. Gum Arabic, extracted from *Acacia Senegal*, is one of the most used anionic polysaccharides for complex coacervation due to its high solubility, excellent emulsifying properties, and ability to form stable colloidal systems [4,5]. However, due to the animal origin of gelatin, there is nowadays a growing demand for alternative plant-based polymers. In recent years, some studies focused on the use of vegetal-derived proteins such as hemp, starch [6,7] or legume proteins [8,9]; however, some proteins are known as allergens and may not be suitable for all applications [10]. Chitosan is a widely known cationic polysaccharide composed of *N*-acetylglucosamine units that can be obtained from the deacetylation of chitin, a biopolymer present in the exoskeleton of crustaceans and the cell walls of fungi. Considered a biocompatible and biodegradable material, chitosan has gained considerable attention due to its multifunctional properties such as antimicrobial activity, antioxidant activity, and film-forming ability [11]. This biopolymer has

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already been studied in the literature for its potential for complex coacervation in combination with gum Arabic [12–14]. However, due to its animal origins, the use of chitosan is subject to controversy. Nevertheless, fungal chitosan, derived from fungi, is a relatively new and promising alternative to traditional chitosan. Fungal chitosan offers several advantages over traditional chitosan, being allergy-friendly and vegan-friendly, including a higher degree of deacetylation, low molecular weight, free of heavy metals, and reduces the reliance on animal sources. Thus, it makes it a more sustainable and environmentally friendly option [15,16].

Currently, limited studies have focused on the use of fungal chitosan combined with gum Arabic for complex coacervation to encapsulate peppermint oil [17] and hexylsalicylate [18], but these systems involve the use of surfactants and additives. Thus, further investigation is necessary to comprehensively comprehend and enhance the development of coacervates using these biopolymers, and to assess their potential for practical applications across diverse industries. For this purpose, α -tocopherol, an active ingredient corresponding to the principal form of vitamin E, was used as a model compound. This vitamin, as the primary lipophilic antioxidant in humans, is frequently utilized in food, pharmaceuticals, and has been incorporated into dermatological goods for over 50 years. Due to its antioxidant properties, which are ascribed to its aromatic hydroxyl group, α -tocopherol functions as an anti-inflammatory agent, reducing the incidence of erythema and sunburn associated with UV radiation exposure [19,20]. Moreover, its topical treatment is acknowledged to enhance skin elasticity, structure, and appearance by firming and hydrating the epidermis and dermis. Nonetheless, its poor solubility in aqueous media and sensitivity to external factors (light, heat, oxygen) limits its application and bioavailability [8]. Although some research has examined the encapsulation of α -tocopherol through complex coacervation, the majority employ animal-derived biopolymers such as chitosan and gelatin [21–23].

The current research aims to explore the formation of complex coacervates between fungal chitosan and gum Arabic in order to identify the most favourable conditions for their interaction and the development of stable coacervates. The study thoroughly analyses the impact of the FC-GA ratio on the morphology, particle size, and rheological properties of wet coacervates. Furthermore, this work offers a detailed characterisation of dry FC-GA coacervates, including their structure, thermal stability, and wetting properties in different media, to achieve a comprehensive understanding of potential applications. To address the existing gaps in the literature, these fully non-animal FC-GA systems were investigated for the encapsulation of α -tocopherol and their encapsulation efficiency, yield, and loading were characterised.

2. Materials and methods

2.1. Materials

Fungal chitosan (FC) of deacetylation degree: 80.9 ± 0.1 % (as determined using conductimetric dosing) and average molecular weight: 39 ± 1 kDa was purchased from Kraeber & Co GmbH (Ellerbek, Germany) and gum Arabic Senegal (GA) (average molecular weight: 393 ± 23 kDa was kindly gifted by Alland&Robert (France). The average molecular weight of both polymers was determined using intrinsic viscosity measurements using Mark-Houwink-Sakurada parameters reported in the literature by Kasai [24] for chitosan and by Gómez-Díaz et al. [25] and Idris et al. [26] for gum Arabic. α -tocopherol (TOCO) $\geq 95\%$ was purchased from Sigma Aldrich (Germany), DUB DIPA (diisopropyl adipate) and DUB SO (ethyl hexyl stearate) were kindly offered by Stéarinerie Dubois (France), glycerol and sweet almond oil were purchased from Aromazone (France). Isohexadecane, hydrochloric acid (HCl), sodium hydroxide (NaOH), and glacial acetic acid were purchased from Fisher Scientific (USA). Absolute ethanol (EtOH) was bought from Brabant (France).

2.2. Preparation of biopolymer stock solutions

Biopolymer stock solutions were prepared by dissolving FC in acetic acid (1%v/v) and GA in deionised water under magnetic stirring for 4 and 2 h, respectively. The biopolymer solutions were kept overnight at 4 °C to ensure complete polymer hydration. The concentration of biopolymer solutions varied from 0.05 to 10 % (w/w).

2.3. Preparation of FC-GA mixtures for ζ -potential and turbidity analyses

Solutions were prepared based on Rousi et al. [27] protocol in order to investigate the impact of the weight mixing ratio R (FC:GA, w/w) and the pH of the solution on the interactions between both polysaccharides. Appropriate amounts of stock solutions were mixed at a final concentration of 0.05 % w/w under magnetic stirring for one hour at room temperature. Biopolymer stock solutions were combined to reach the following FC:GA ratios: 1:1, 1:2, 1:3, 1:4, 1:5, and 1:7. The pH was adjusted from 3.0 to 7.0 with an increase of 0.5 units of pH by using HCl or NaOH solutions with a concentration between 0.1 and 2 M. Both FC and GA solutions were used as blanks under the same conditions.

2.3.1. ζ -potential measurements

The electrical surface charge of particles was investigated using Zetasizer 5000 (Malvern Instruments GmbH, Germany) using DTS1070 cells at 25 °C. The ζ -potential (ζ , mV) values were calculated by using the device software and applying the Helmholtz-Smoluchowski model (Eq.1):

$$\zeta = 4\pi\mu\eta/D \quad (1)$$

where, μ is the electrophoretic mobility, D is the dielectric constant of the dispersant in the boundary layer, and η is the viscosity. Measurements were conducted in triplicate for each sample, with three analyses per measurement. The strength of electrostatic interactions (SEI) between the biopolymers was calculated based on the following equation described by Weinbreck et al. [28] (Eq. 2):

$$SEI(mV^2) = |ZP_1 \times ZP_2| \quad (2)$$

2.3.2. Turbidity measurements (UV-vis)

Turbidity (T , cm^{-1}) of stock solutions and prepared mixtures was determined by measuring the absorbance at 600 nm using a UV / VIS spectrophotometer Shimadzu UV-1800 (Shimadzu, Duisburg, Germany) with a 1 cm path length quartz cuvette. Turbidity was determined using the following equation (Eq.3):

$$T = -\left(\frac{1}{L}\right) \ln\left(\frac{I}{I_0}\right) \quad (3)$$

where L is the path length (cm), I and I_0 correspond to the light intensity on the detector in the presence of sample or water, respectively. All measurements were performed in triplicate at room temperature.

2.4. Preparation of FC-GA complex coacervates

The stock solutions of FC (5 % w/w) and GA (10 % w/w), were combined in specific FC:GA ratios (w/w) to reach a total biopolymer concentration of 5 % w/w. The pH of the obtained mixtures was adjusted using HCl or NaOH solutions from 0.1 to 2 M to be equal to their optimum pH (pH_{opt}). Resulting mixtures were stirred for 30 min after pH adjustment and left at rest for 24 h at room temperature. After 24 h, the polymer mixture was filtered through Whatman no. 42 filter paper using a Büchner funnel. The resulting sample is here referred to as “liquid coacervates”. The liquid coacervates were then placed into Petri dishes for freeze-drying during (-96 °C) to obtain a powder by grinding the frozen coacervates. The resulting powder is referred to as “dry coacervates”.

2.5. Encapsulation of α -tocopherol (FCGA-TOCO coacervates)

To evaluate the potential of FC-GA coacervates for encapsulation purposes, α -tocopherol was selected as a lipophilic active ingredient. FCGA-TOCO coacervates were prepared by selecting the optimised parameters described in 3.1 (ratio FC:GA 1:4 w/w and pH 4.8) as follows:

- α -tocopherol was dissolved in diisopropyl adipate at 10 % w/w under magnetic stirring,
- The oil phase was emulsified into a solution of GA 5 % w/w using a VMI Turbotest (Turbotest, VMI-mixing, La-Roche-sur-Yon, France) equipped with a deflocculator turbine (diameter of 35 mm) operating at 1000 rpm for 10 min,
- The resulting emulsion was stirred at 8000 rpm for 5 min using a rotor-stator blender T25 digital ultra-turrax equipped with turbine S25 N—25F (IKA, Freiburg, Germany),
- The FC solution (5 % w/w) was gradually added to the emulsion under magnetic stirring,
- After 10 min, the entire FCGA-TOCO solution underwent dilution to reach 5 % of total solid content, and continued magnetic stirring for another 10 min,
- Finally, pH adjustment was performed to reach optimal levels (4.8), followed by maintaining magnetic stirring for 30 more minutes before keeping the solution at rest for 24 h at room temperature,
- After 24 h, polymer mixture was filtered through Whatman no. 42 filter paper using a Büchner funnel. Subsequently, liquid coacervates were placed into Petri dishes for freeze-drying during 4 h ($-96\text{ }^{\circ}\text{C}$) to obtain a powder by grinding the frozen coacervates.

2.6. Characterisation of liquid coacervates

2.6.1. Morphology and particle size of FC-GA coacervates

Liquid coacervates microstructure was assessed by phase-contrast microscopy using an optical microscope ECLIPSE NI-U Nikon (Nikon, Japan) equipped with a camera DS-Fi3 (5.9 megapixels CMOS image sensor) at a magnification ranging from x100 to x400. All observations were performed at room temperature.

Particle diameters were determined by static light scattering using a Shimadzu SALD-7500-Nano particle size analyser (Shimadzu, Duisburg, Germany) and Wing SALD II Version 3.1.0. After a 30-min delay following pH adjustment, liquid coacervates were first dispersed in deionised water and stirred during the analyses. Samples were analysed in triplicate, and the results were expressed as average values D[50].

2.6.2. Rheological measurements

The rheological properties of FC-GA liquid coacervates were assessed with a DHR-2 hybrid rheometer (TA Instruments, USA) using an aluminium cone-plate devices with a diameter of 40 mm and a cone truncation of 54 μm and a cone angle of 2°. Temperature was controlled at 20 $^{\circ}\text{C}$ using a Peltier plate.

Shear flow experiments were carried out using a continuous flow ramp with shear rates from 10^{-3} to 1000 s^{-1} and inversely for five minutes. Data are represented as shear viscosity as a function of shear rate.

Elastic moduli (G') and loss moduli (G'') were measured using strain amplitude sweep tests at a constant frequency of 1 Hz at 20 $^{\circ}\text{C}$.

2.6.3. Thixotropy

To evaluate the thixotropic properties of FC-GA coacervates, alterations of low (10^{-3} s^{-1}) and high (1000 s^{-1}) shear rates were applied for 1 min each for a total time of 5 min. The resulting viscosity was displayed as a function of time.

2.7. Characterisation of dry FC-GA coacervates

2.7.1. Fourier transform infrared spectroscopy (FT-IR)

Chemical analyses of FC-GA dry coacervates were performed by FT-

IR using a PerkinElmer Frontier FT-IR spectrometer (PerkinElmer, USA). The transmittance of the samples was recorded by mixing microparticles with potassium bromide (KBr) in the range 4000–650 cm^{-1} with an average of 32 scans at a resolution of 4 cm^{-1} . Measurements were performed at room temperature.

2.7.2. Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC 8000, Perkin-Elmer, USA) was used to determine dry coacervates' glass transition temperature (T_g). The sample (5 mg), was placed in an aluminum pan with a hole and analysed under a nitrogen gas flow of 20 mL/min. Samples were analysed at 10 $^{\circ}\text{C}$ per minute within a temperature ranging from 25 $^{\circ}\text{C}$ to 250 $^{\circ}\text{C}$.

2.7.3. Thermal gravimetric analyses (TGA), derivative thermogravimetric analyses (DTG) and moisture content

Thermal properties of FC-GA coacervates were evaluated using Set-sys TGA 1200, SETARAM (Caluire-et-Cuire, France) apparatus from 25 $^{\circ}\text{C}$ to 800 $^{\circ}\text{C}$ in air atmosphere. Moisture content was determined by measuring the total weight loss up to 150 $^{\circ}\text{C}$.

2.7.4. Contact angle measurements

The contact angle was measured using a DSA30E KRÜSS contact angle goniometer equipped with a plastic syringe containing deionised water. Tablets (100 mg) made from dried FC-GA coacervates using a pellet press with a pressure of five tons (Fritsch GmbH, Germany) were placed on the platform of the equipment and a 7 μL droplet was deposited on the surface at a rate of 1 $\mu\text{L/s}$ at 20 $^{\circ}\text{C}$. The camera on the device recorded images of the droplet, and the software within the device automatically calculated the contact angle. Five tablets per FC:GA ratio were prepared and one droplet was applied to each tablet. The reported contact angle value corresponds to an average of 10 measurements taken at one-second intervals for each droplet.

2.7.5. Determination of wettability index

Determination of the wettability index was performed according to the method adapted from Jinapong et al. [29]. A 250 mL glass beaker was filled with 100 mL of solution at room temperature. A glass funnel was placed on a ring stand approximately 10 cm above the liquid surface. To obstruct the opening of the funnel, a test tube was placed inside and surrounded by 0.1 g of FC-GA (with a ratio of 1:4). The timer started as soon as the tube was lifted, and the time it took for the powder to fully wet (with water penetrating its surface) was recorded.

The wettability index of the powder was determined by evaluating its behaviour in different solvents, including isohexadecane (apolar), ethyl hexyl stearate (ester), glycerol (polyol), sweet almond oil (vegetal oil), ethanol, and water (polar protic solvents). Samples were then observed by optical microscopy to evaluate the impact on the coacervates' microstructure.

2.7.6. Encapsulation efficiency and active loading

Encapsulation efficiency (EE) and active loading (AL) of FCGA-TOCO were evaluated as follows: 100 mg of sample were weighed in a plastic tube with 10 mL of pure EtOH (in triplicate). The solution was placed in an ultrasonic bath (VWR Ultrasonicator) for 10 min at room temperature to extract the encapsulated α -tocopherol. The solution was then filtered using a 0.45 μm pore-sized cellulose acetate filter. A standard calibration curve ($R^2 = 0.999$) was used, with the α -tocopherol concentration determined by calculating the area under the UV detection peak after separation by LC-MS.

The concentration of α -tocopherol in coacervates (AL) was measured using an LC-MS Agilent 1200 (Agilent Technologies, Waldbronn, Germany) equipped with an C18 XDB 5 μm 6.6 \times 150 mm column and a UV-vis detector Agilent Infinity 1260 VL+. The compound was eluted in a mobile phase milliQ water/MeOH at ratio 2/98 in isocratic mode at a

flow rate of 1.5 mL/min. The column temperature was maintained at 40 °C and the detection wavelength was 290 nm. The elution time of α -tocopherol was 4.7 min.

The encapsulation efficiency (EE) was calculated as the ratio between the experimental concentration and the theoretical concentration of α -tocopherol in the coacervates (Eq. 4):

$$EE(\%) = \frac{[\alpha - \text{tocopherol}]_{\text{exp}}}{[\alpha - \text{tocopherol}]_{\text{theo}}} \times 100 \quad (4)$$

2.8. Statistical analyses

All analyses were conducted at least in duplicate. The results are presented as the mean \pm standard deviation (SD). Data collected were analysed by one-way ANOVA and compared with Tukey's HSD test at a confidence level of 95 % using XLSTAT software (Version 2016.02.27444, Addinsoft, Paris, France).

3. Results and discussion

3.1. Identification of optimal parameters for complex coacervation of FC and GA

Complex coacervation is mainly driven by electrostatic interactions between two oppositely charged molecular species that undergo associative phase separation, forming a concentrated phase containing both species. This association is highly dependent on the environmental pH, as the latter is optimal when the charge difference between the two polymers is maximum [2]. The optimal pH range for complex coacervation was determined by assessing the ζ -potential of individual polymers as a function of pH (3.0–7.0) (Fig. 1).

According to Fig. 1, it was observed that GA showed a negative charge across all pH levels considered. This can be attributed to the carboxyl groups (COO^-) within the polymer structure when the pH is greater than 2.2, corresponding to the GA pKa value [28]. However, FC, known as a cationic polysaccharide, exhibited a positive charge throughout the pH range of 2–6 as a result of protonation of its amino groups ($-\text{NH}_2$), the latter having a pKa of 6.0. [11]. The charge

difference between GA and FC allows electrostatic interactions, thus leading to the formation of complex coacervates.

The optimum pH for complex coacervation is defined as the pH at which the strength of electrostatic interactions between two polymers is maximised, resulting in the strongest coacervation complex. The strength of the electrostatic interaction (SEI) is calculated as the product of the absolute value of ζ -potential of the two polysaccharides at each pH value [28,30]. For the FC-GA system, the strongest SEI was observed between pH 4 and 5, with a maximum at pH 5. Similar results were reported by Espinosa-Andrews et al. [30] for an animal chitosan-GA system.

The ideal weight ratio of FC to GA can be determined by measuring the pH at which electrostatic interactions are strongest, typically around 5. To identify the isoelectric point (IP) of each mixture and track the formation of coacervates with varying pH values, ζ -potential and turbidity measurements were performed for different FC:GA ratios. The resulting curves illustrate how IP and coacervate formation vary as a function of pH for the different weight ratios studied (Fig. 2).

For the same FC:GA ratio (w/w), increasing the pH resulted in a decrease in the zeta potential of the mixture. This phenomenon may be attributed to the neutralisation of amino groups in chitosan and excess carboxyl groups in gum Arabic, which reduces the overall positive charge [27].

Results from the turbidity measurement align with the zetametry findings, indicating that the maximum turbidity is achieved when the pH of the mixture matches the isoelectric point (IP). These observations led us to determine an ideal FC:GA ratio of 1:4 (w/w), with IP at pH 5. Similar ratios were reported in previous studies by Butstraen & Salaün [12] and Espinosa-Andrews et al. [31] who proposed optimal chitosan:GA ratios between 1:4 and 1:5 (w/w). These parameters are especially appropriate for topical cosmetic products, which have a pH approximately equal to that of the skin (≈ 5). As the process of complex coacervation is primarily impacted by pH and ionic strength, special care must be taken to final applications that may contain salt or be prepared at varying pH levels, as these factors may induce structural changes which may lead, in these extreme cases, to destabilization or even disintegration of the coacervates and, consequently, premature release of the active component.

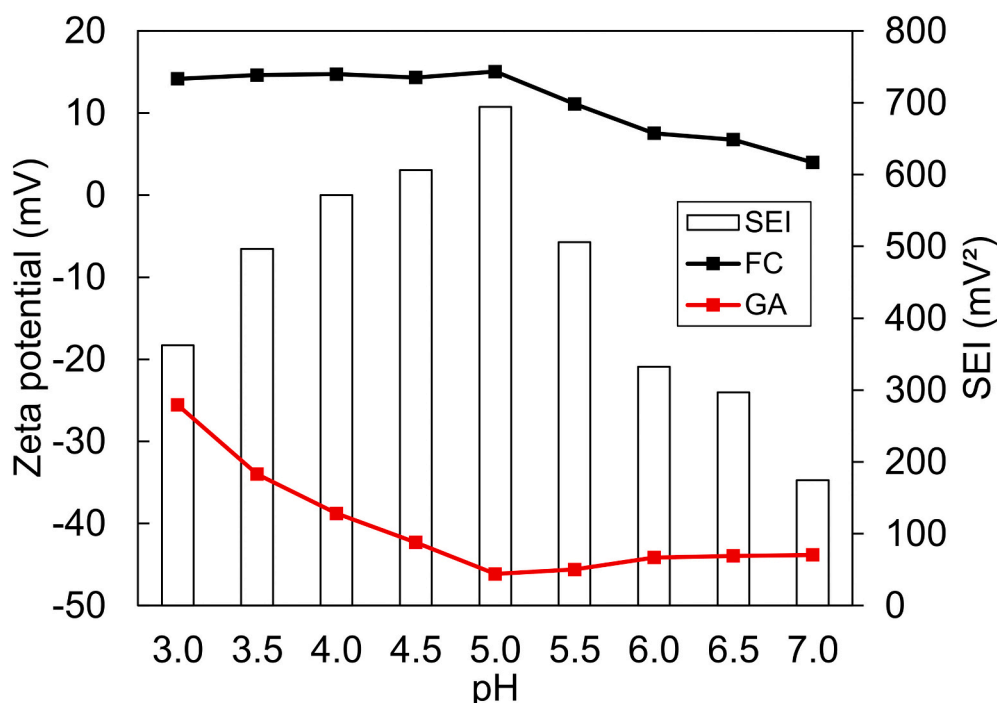


Fig. 1. Impact of the pH on the ζ -potential of fungal chitosan (FC) and gum Arabic (GA) stock solutions, depending on the pH.

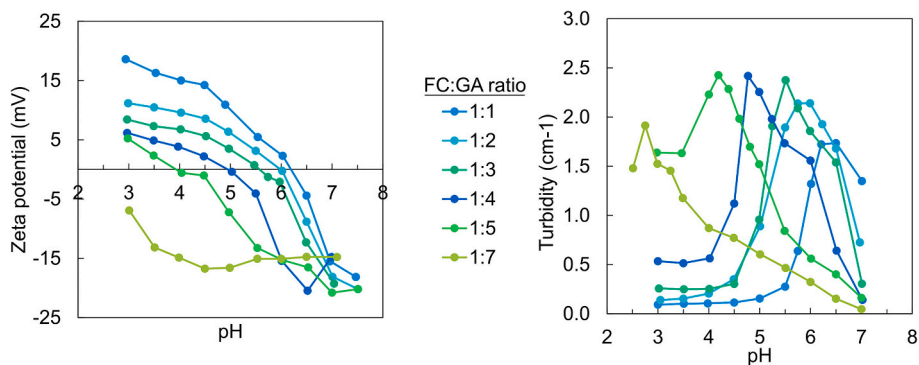


Fig. 2. ζ -potential measurements (a) and turbidity analyses (b) of fungal chitosan (FC)-gum Arabic (GA) mixtures, depending on the pH.

3.2. Effect of the weight ratio on the morphology of coacervates

FC-GA coacervates were formed at different ratios of FC:GA w/w and a total polymer concentration of 5 % w/w, and their morphology was examined by phase-contrast microscopy and particle size analyses after dilution in deionised water. The results are shown in Fig. 3, with the particle sizes indicated as the volume median diameter D[50], representing the volume at 50 % v/v on the distribution curve. The coacervate structure appeared to be more aggregated with larger particles at a ratio

of 1:4 ($101.5 \pm 11.0 \mu\text{m}$), while smaller particles were observed at ratios of 1:1 ($8.5 \pm 2.6 \mu\text{m}$), 1:2 ($5.2 \pm 0.6 \mu\text{m}$), and 1:7 ($33.5 \pm 5.0 \mu\text{m}$). These last three corresponding coacervates were formed at pH values of 6.5, 6, and 2.8 respectively, far from the optimal pH range identified in 3.1 (i.e., pH 4.5–5). Furthermore, the maximum turbidity and SEI values were also lower at these pH values than the optimal pH range, indicating less efficient coacervate formation [32]. As expected, a strong correlation was observed between theoretical SEI and actual particle size (Pearson correlation coefficient, $r = 0.96$; $p < 0.02$). Consequently,

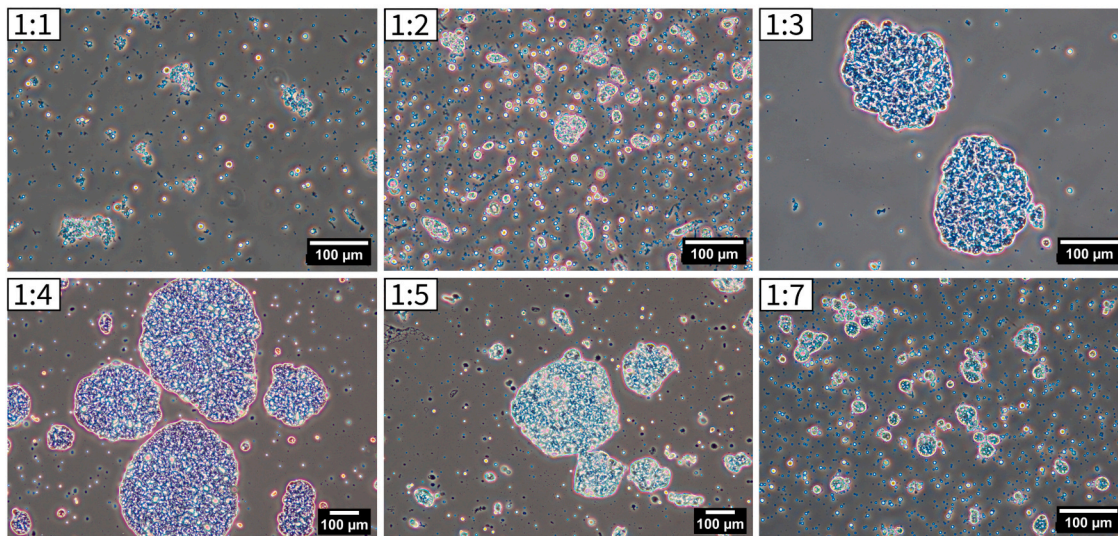
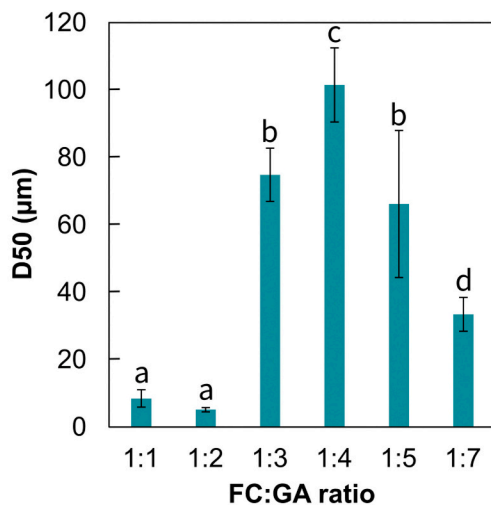


Fig. 3. Median diameter in volume and phase-contrast microscopy images of fungal chitosan (FC)-gum Arabic (GA) coacervates formed with varied weight ratios.

these results clearly indicate that SEI may be an important indicator of coacervate formation, with higher SEI values resulting in larger coacervate particles.

3.3. Rheology measurements

Fig. 4 shows the flow curves (a) and oscillatory behaviour (b) of liquid coacervates with different ratios of FC:GA (w/w). All samples exhibited shear thinning behaviour with an increase in the shear rate to approximately 10 s^{-1} (Fig. 4a). Ratio 1:1 showed the highest viscosity among all tested ratios. It should be noted that, at this specific ratio, the macroscopic appearance of the coacervates was suspension-like, without a dense sedimented coacervate phase, so contrary as usually observed. This may be because the pH of the solution (6.5) is close to the pKa of fungal chitosan, resulting in solid chitosan particles remaining in the coacervate phase, leading to higher viscosity. This can also be observed in Fig. 4b, where the storage modulus G' is higher than the loss modulus G'' at low amplitudes, indicating that the ratio 1:1 behaves more as a solid with a gel-like behaviour.

For all samples, a sharp decrease in viscosity was observed at high shear rates (above 10 s^{-1}), indicating that the coacervates experienced structural breakdown under these conditions. This important phenomenon has been reported in multiple investigations of complex coacervates [33–35]. Fig. 4b shows the linear viscoelastic region as evidenced for all coacervates, which refers to the amplitude range that can be applied to a sample without causing disturbing, destruction, or breakdown. Interestingly, this indicates the deformability of the sample. The 1:4 ratio exhibited a larger LVER (linear viscoelastic region), suggesting higher deformability compared to the other ratios tested. This can be attributed to the stronger electrostatic interactions present at this ratio. This viscoelastic behaviour is desirable for various applications because it enables easy flow and deformation during processing while preserving the stability and integrity of the coacervate structure.

To gain a deeper understanding of the microstructure alterations and the resulting decrease in viscosity under shear, phase-contrast microscopy observations of samples were carried out at different stages: initially when submitted to low shear [0.001 s^{-1} (A)], immediately after maximum shear [1000 s^{-1} (B)] and finally once at low shear [0.001 s^{-1} (C)] for a ratio of 1:4 (FC:GA), as shown in Fig. 5. To the best of our knowledge, this is the first time such a complementary approach has been conducted in order to enhance the understanding of the reversibility phenomena of coacervates.

At a shear rate of 0.001 s^{-1} , the coacervate particles appeared well-defined with a spherical shape and minimal aggregation, indicating a stable and structured coacervate phase. However, once subjected to a

shear rate of 1000 s^{-1} , the coacervate particles became highly distorted, suggesting significant disruption of the coacervate structure under high shear conditions. These observations suggest that shear forces can cause deformation of coacervate structures, leading to a sharp viscosity reduction without causing complete breakdown or destruction of the coacervate phase. To our knowledge, this is the first study to investigate the effect of shear rate on the microstructure of non-animal coacervates.

The ability of the coacervates to return to their original state was examined using a reversibility test. This test involved subjecting the coacervates to alternating high- and low-shear cycles (Fig. 6).

These results illustrate that coacervates exhibited reversible behaviour, as evidenced by their ability to recover their initial structure after undergoing cycles of high shear followed by low shear. This reversibility indicates the excellent ability of the FC-GA coacervates to recover their initial structure through electrostatic interactions, further highlighting their potential for encapsulation purposes [36]. The observed reversible behaviour aligns with prior research on complex coacervate systems, including β -lactoglobulin/lactoferrin [34] and gelatin/gum Arabic [35]. This phenomenon was attributed to weak electrostatic interactions that allow the structure to recover with the decrease in shear rates. To the best of our knowledge, this study is the first to explore the impact of shear rate on the microstructure and reversibility of FC-GA coacervates, gaining a deeper understanding of the reversibility phenomena. Observations clearly highlight the promising potential for the use of such encapsulating systems in a variety of applications involving high shear rates, such as sampling, mixing, extrusion, or formulation processes.

3.4. Characterisation of dry FC-GA coacervates

3.4.1. Infrared spectroscopy analyses (FT-IR)

The FT-IR spectra of the individual polysaccharides FC, GA, and FC-GA coacervates (ratio 1:4 w/w) are displayed in Fig. 7. FC exhibited a wide band at approximately 3400 cm^{-1} , which is attributed to the O–H stretching vibration of the hydroxyl groups. Additionally, characteristic peaks were also observed at 1647 cm^{-1} corresponding to the stretching vibrations of the carbonyl group and 1598 cm^{-1} and 1421 cm^{-1} to the N–H bending vibration of the amine groups and the C–N stretching vibration in the amide group, respectively. Analyses of the GA spectrum showed peaks at 3400 cm^{-1} for O–H stretching and at 1609 cm^{-1} and 1420 cm^{-1} for the asymmetric and symmetric stretching vibrations of the carboxyl groups, respectively. FTIR examination of the FC-GA coacervates revealed alterations in the N–H bending vibration, which shifted from 1598 to 1537 cm^{-1} . Furthermore, there was increased absorption of the peak at approximately 1414 cm^{-1} . These alterations in

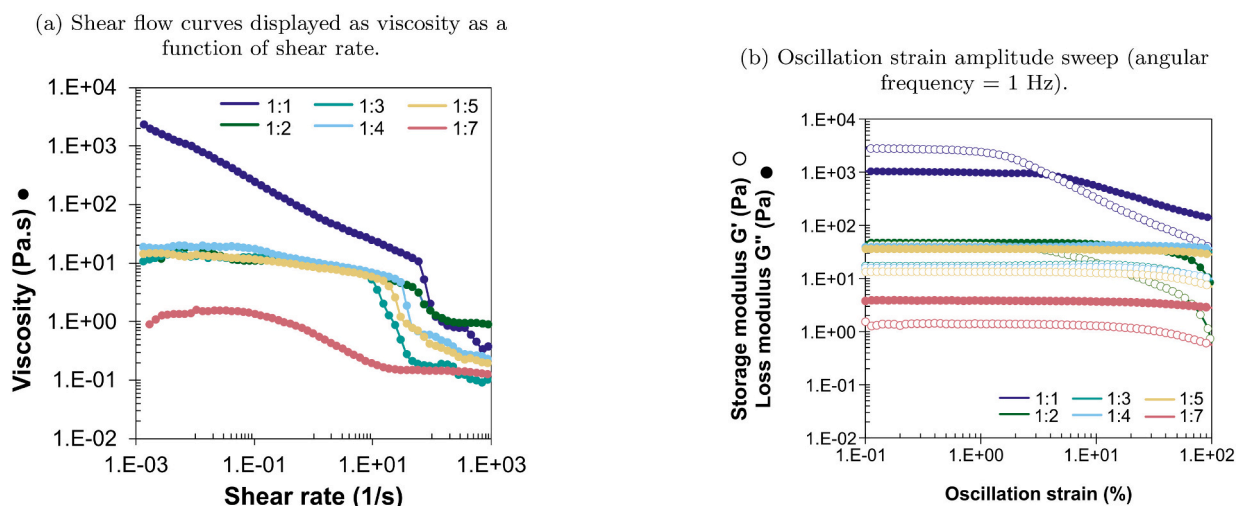


Fig. 4. Rheological behaviour of liquid fungal chitosan (FC)-gum Arabic (GA) coacervates from ratio 1:1 to 1:7 (FC:GA w/w).

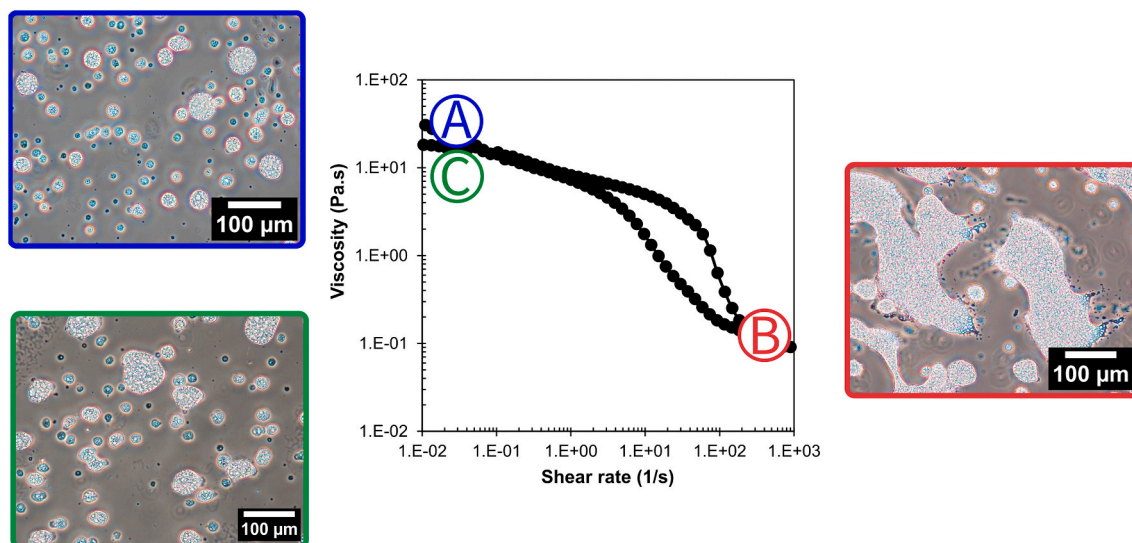


Fig. 5. Fungal chitosan-gum Arabic liquid coacervates shear flow curve coupled with phase-contrast microscopy observations at rest 0.001s^{-1} (A), high shear 1000s^{-1} (B) and at rest after shear 0.001s^{-1} (C).

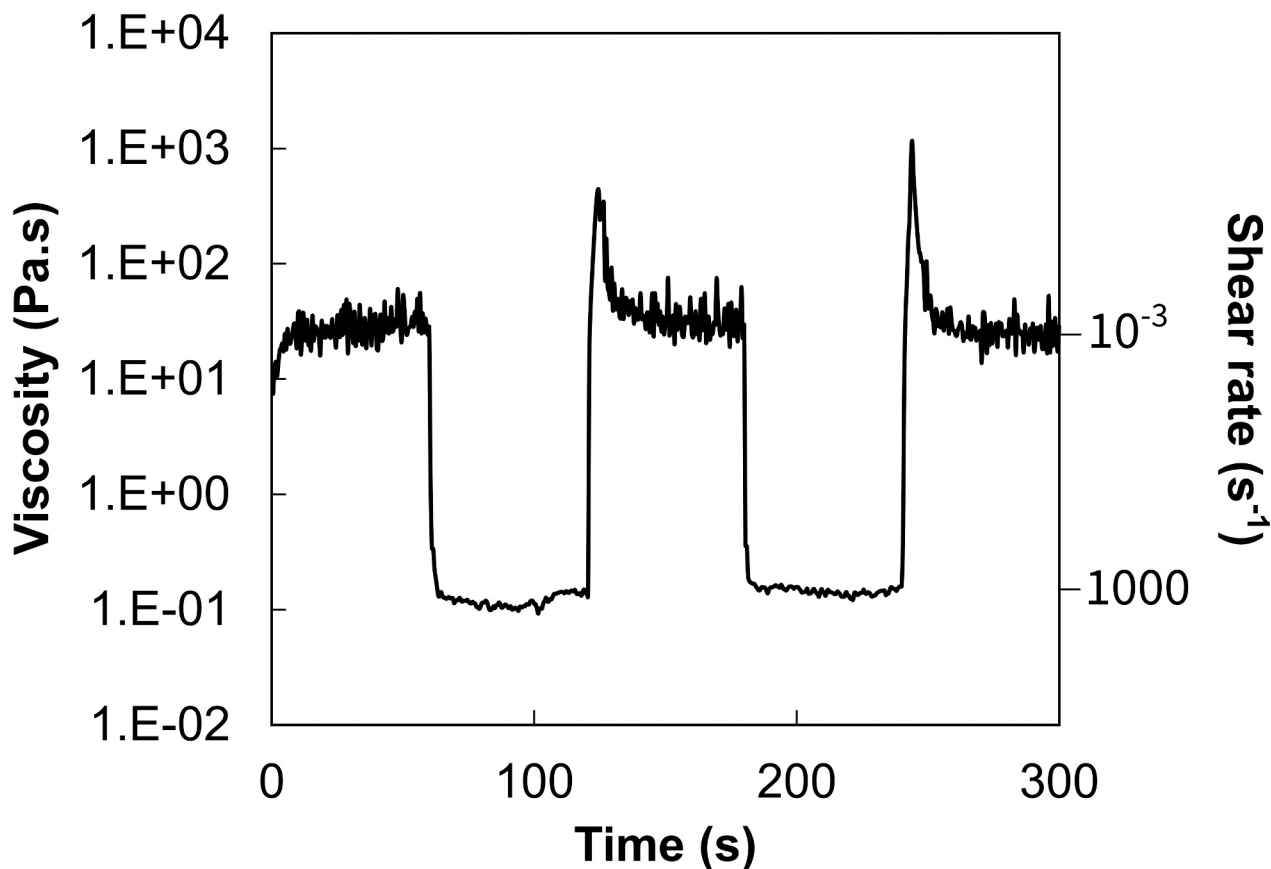


Fig. 6. Thixotropy properties of fungal chitosan (FC)-gum Arabic (GA) coacervates. Steps were alternated between low (0.001s^{-1}) and high (1000s^{-1}) shear rates (1 min each for a total period of 5 min).

the FTIR spectra undoubtedly evidence the occurrence of electrostatic interactions between FC and GA within the coacervate structure [13,37,38].

3.4.2. Differential scanning calorimetry analyses

DSC thermograms of pure FC, GA, and FC-GA coacervates are displayed in Fig. 8. Broad endothermic peaks were detected at $74\text{ }^{\circ}\text{C}$ and

$78\text{ }^{\circ}\text{C}$ on the thermograms of pure FC and GA, respectively, representing their corresponding glass transition temperatures. Previous studies by Niu et al. [33] and Ren et al. [39] have also reported similar Tg values for these polysaccharides. Upon complex coacervation between FC and GA, a Tg shift towards higher temperatures (around $80\text{--}90\text{ }^{\circ}\text{C}$) was observed, indicating the occurrence of a new structured network thus enhancing thermal stability, requiring greater energy to disrupt the

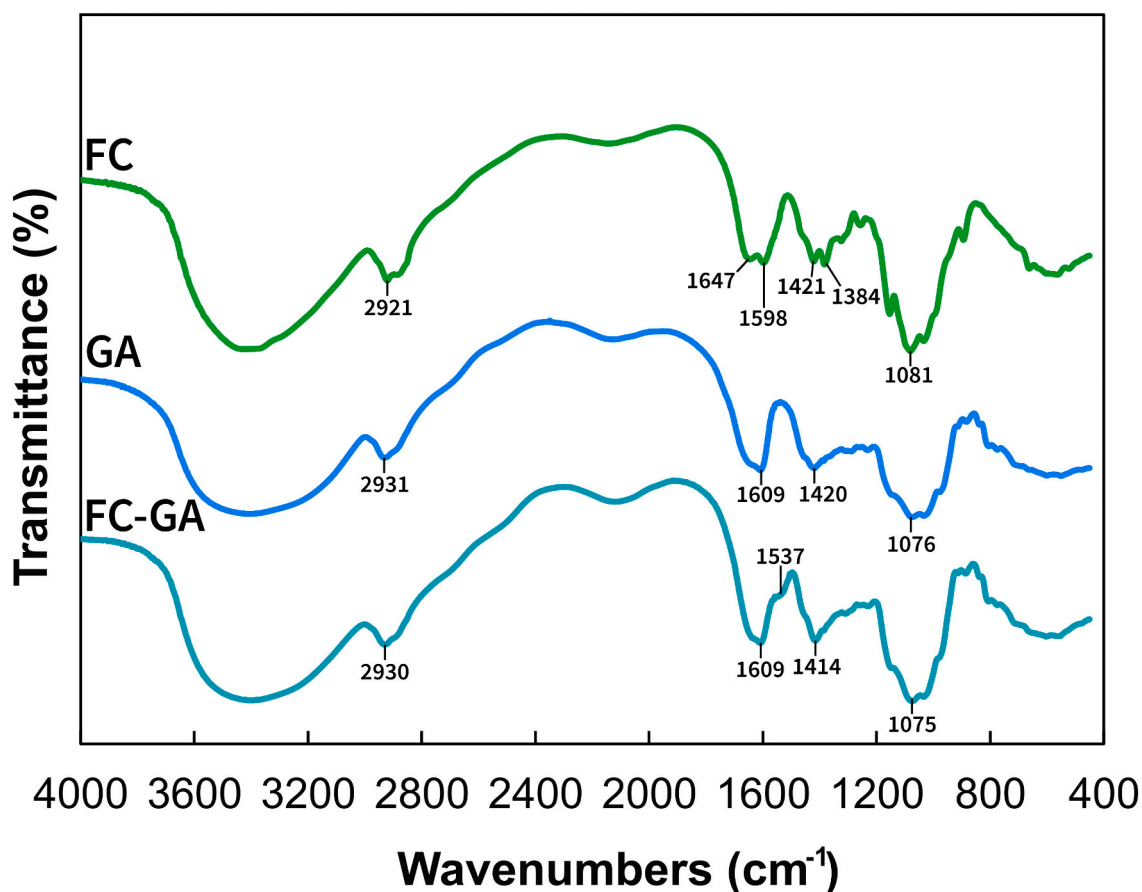


Fig. 7. FTIR spectrum of fungal chitosan (FC), gum Arabic (GA), and FC-GA dried coacervates.

coacervate structure, as noted by Huang et al. [40] and Yücepete et al. [41]. Consequently, these formed coacervates exhibit enhanced thermal stability compared to individual polysaccharides such as FC and GA, making them well-suited for applications involving high temperatures.

3.4.3. Thermogravimetric analysis of FC-GA coacervates (TGA)

TGA was used to evaluate the thermal stability of the individual polysaccharides and FC-GA coacervates (Fig. 9). As similar thermal behaviours were found for all ratios, a 1:4 ratio was chosen to improve visual clarity. FC and GA showed a 3-step degradation process consisting of water desorption and evaporation below 150 °C, followed by polysaccharide decomposition within the 250–400 °C range, and finally residual carbonated group decomposition above 400 °C. A similar behaviour was observed for all FC-GA coacervates, with minor variations in the temperature ranges for each degradation step. Prior studies [5,38,42,43] have reported comparable thermal characteristics for individual polymers and animal-sourced chitosan-GA coacervates. These findings clearly confirm the robustness of the thermal stability of the FC-GA coacervates, making them suitable for high-temperature processes.

3.4.4. Contact angle and wettability index

Once the sample were freeze-dried, powder tablets were prepared using FC-GA coacervates with different FC:GA ratios to evaluate their hydrophilic/lipophilic characteristics by measuring the contact angle of a water droplet deposited onto the surface. The average contact angles of the five tablets (with 10 measurements/tablet) are shown in Fig. 10. The contact angle values ranged from 64.8° for the lowest ratio of 1:1 to 44.8° for the highest ratio of 1:7, showing that the contact angle decreased with an increase in the ratio of FC to GA. Notably, although FC is insoluble at neutral pH, GA remains water-soluble with the whole pH range investigated. Therefore, lowering the FC-GA coacervates

enhanced their hydrophilicity and reduced their contact angle, indicating improved wettability. This observation aligns with a recent study by Han et al. [44] which reported a contact angle value of 58° for a chitosan-gum Arabic system at a ratio of 1:5 (w/w). This trend was similarly noted by Lobato-Guarnido et al. [45] when the proportion of GA in these coacervates was increased.

To explore interactions with different media, the wettability index of FC-GA (1:4 w/w) was measured in different usual cosmetic solvents owning different physicochemical properties: isohexadecane, ethyl hexyl stearate, glycerol, sweet almond oil, ethanol, and water. As defined by Ipar et al. [46], powders are considered wettable if their wetting time is less than 60 s, while they are classified as non-wettable if they exceed 120 s. The wettability index values of FC-GA coacervates are gathered in Table 1:

Based on the wettability index values, three different behaviours were observed: 1) FC-GA coacervates exhibited excellent wetting properties in isohexadecane, ethanol, and ethyl hexyl stearate with wetting times under 60 s; 2) while moderate wetting properties were observed for sweet almond oil with wetting times of approximately 60 s; 3) conversely, poor wetting properties were observed in water and glycerol, with wetting times exceeding 120 s. These differences can be attributed to the solvents' polarity variation. As reported by Moulik et al. [47], low-polarity solvents may weaken electrostatic interactions.

The dielectric constants of specific and similar solvents are listed in Table 1. Isohexadecane, ethyl hexyl stearate, and ethanol exhibit relatively low dielectric constants, which may enhance the coacervates wetting properties due to weaker electrostatic interactions. In contrast, polar solvents such as water and glycerol may impede the wetting properties of FC-GA coacervates, as they preserve strong electrostatic interactions. The dielectric constant of oleic acid was used for sweet almond oil, since its value was unavailable. However, it is important to

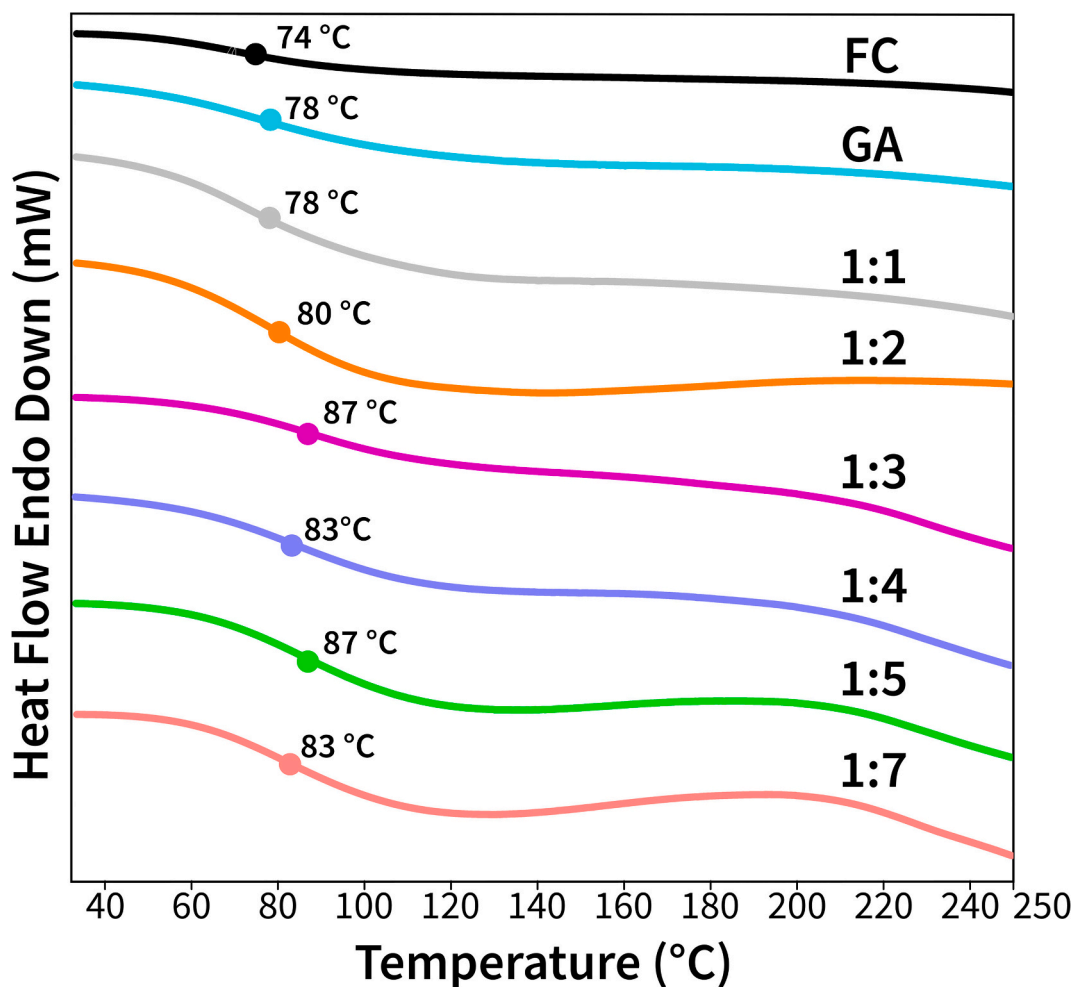


Fig. 8. DSC thermograms of fungal chitosan (FC), gum Arabic (GA), and FC-GA coacervates (5 wt% total biopolymer concentration).

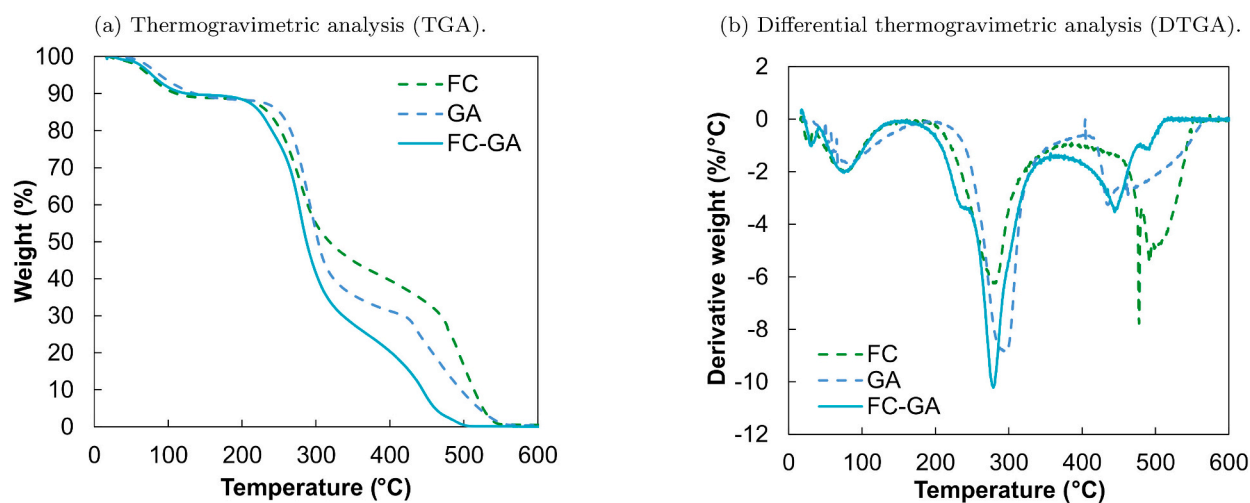


Fig. 9. Thermogravimetric analysis of fungal chitosan (FC)-gum Arabic (GA) coacervates.

note that sweet almond oil has a complex composition containing both unsaturated and saturated fatty acids, vitamins, and other compounds that could affect the wetting behaviour of coacervates differently from pure oleic acid.

Observations of coacervates in solvents were performed using optical microscopy, with the corresponding microphotography shown in

Fig. 11:

Microscopic observations suggest that the stability and dispersion of FC-GA coacervates are influenced by the solvent polarity, with lower dielectric constant solvents causing the deconstruction of coacervates. Higher dielectric constant solvents lead to larger particle sizes and potential aggregation. Therefore, it is important to consider the dielectric

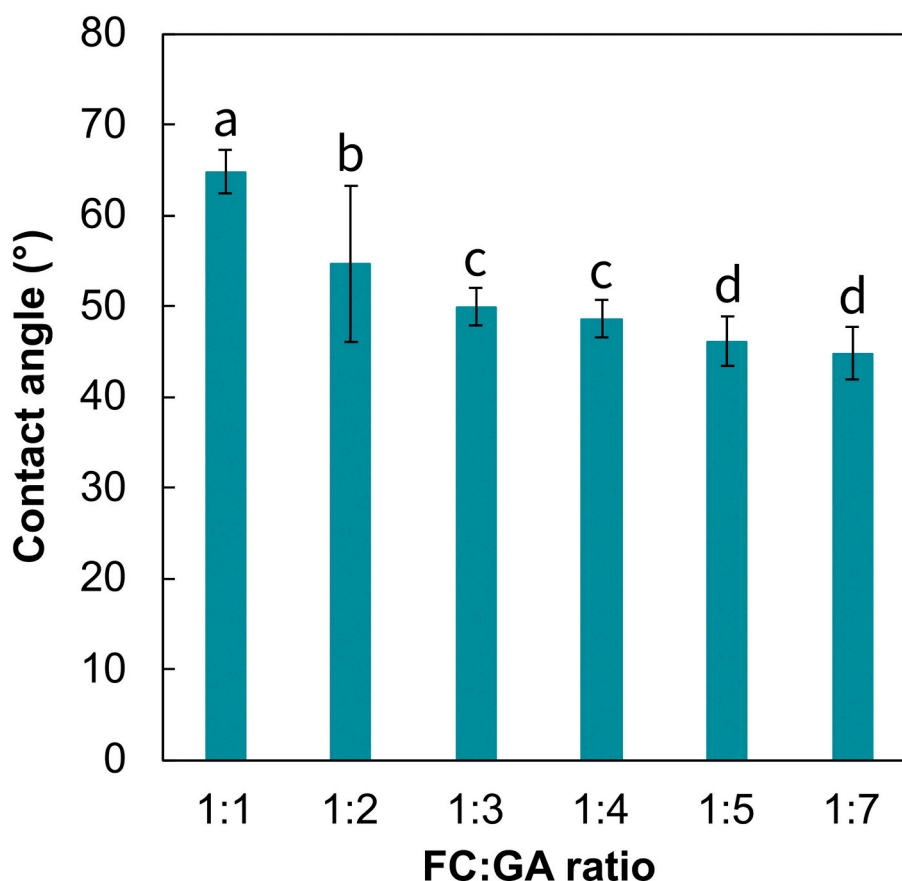


Fig. 10. Contact angles of coacervates formed at different mass ratios FC:GA.

Table 1

Fungal chitosan (FC):gum Arabic (GA) (1:4 w/w) powder's wettability index in various media and their corresponding dielectric constant extracted from Reaxys® database.

Solvent	Wettability index (s)	Dielectric constant (From Reaxys®)
Glycerol	>120 ± 0	46
Water	>120 ± 0	78
Sweet almond oil	61 ± 14	2.6 (oleic acid)
Ethyl hexyl stearate	7 ± 1	2.3 (stearic acid)
Isohexadecane	4 ± 1	2 (hexadecane)
EtOH	2 ± 0	24

constant of the solvent when studying the wetting properties and stability of coacervates for future application.

3.5. Encapsulation of α -tocopherol by complex coacervation

To evaluate the performance of FC-GA coacervates to encapsulate α -tocopherol, a lipophilic active compound, a series of encapsulation steps were carried out using optimised parameters identified previously (i.e., FC:GA 1:4, pH 4.8). Five ratios WM:A (wall-material:active) were tested: 1:1, 3:1, 5:1, 7:1 and 1:2. The concentration of active in the coacervates (active loading) and the encapsulation efficiency (EE) were calculated and reported in Table 2. The active loading refers to the amount of α -tocopherol present within the coacervates. A higher WM:A ratio led to reduced active loading due to its direct correlation with the concentration of active in solution. Furthermore, EE ranged from 70.0 % to 82.6 %, and increasing this ratio did not consistently improve encapsulation efficiency. Therefore, it was determined that the most effective ratio for encapsulating α -tocopherol using FC-GA coacervates was found to be 1:1 (WM:A), resulting in a yield of 79.6 %, an active

loading of 5.27 %, and an encapsulation efficiency of 82 %. Carpentier et al. [8] achieved comparable outcomes with pea protein isolate and taragum (77.4 %), Budinčić et al. [48] reported similar findings using sodium lauryl ether sulfate and chitosan (73.3 %), while Huang et al. [49] used soy protein isolate and chitosan to achieve a result of 64.8 %. These results of encapsulation of α -tocopherol by FC-GA coacervates demonstrate their high potential as an effective encapsulation system for lipophilic active compounds in food, pharmaceutical and cosmetic applications.

4. Conclusion

Fungal chitosan (FC) and gum Arabic (GA) were studied to develop new non-animal complex coacervates for encapsulation. The electrostatic interactions between FC and GA were analysed and the optimal conditions for forming the coacervates were determined at a pH of 5 with an FC:GA weight ratio of 1:4. Rheological assessments demonstrated that the liquid coacervates exhibited shear-thinning behaviour. The innovative complementary approaches such as rheology coupled with phase-contrast microscopy observations indicated that the FC-GA coacervates could undergo structural changes at high shear rates; nevertheless, they fully recovered their original state when shear ceased. These findings suggest that FC-GA coacervates have very promising potential as stable encapsulation materials for processes involving high shear forces, such as formulation in cosmetic or food industries. Furthermore, FTIR, DSC, and TGA analyses allowed checking the presence of electrostatic interactions between these biopolymers and their thermal stability, rendering them suitable for high-temperature procedures, such as spray-drying or food extrusion. In addition to that, the present study demonstrates that increasing the concentration of GA in the FC-GA coacervates increased their hydrophilicity and improved

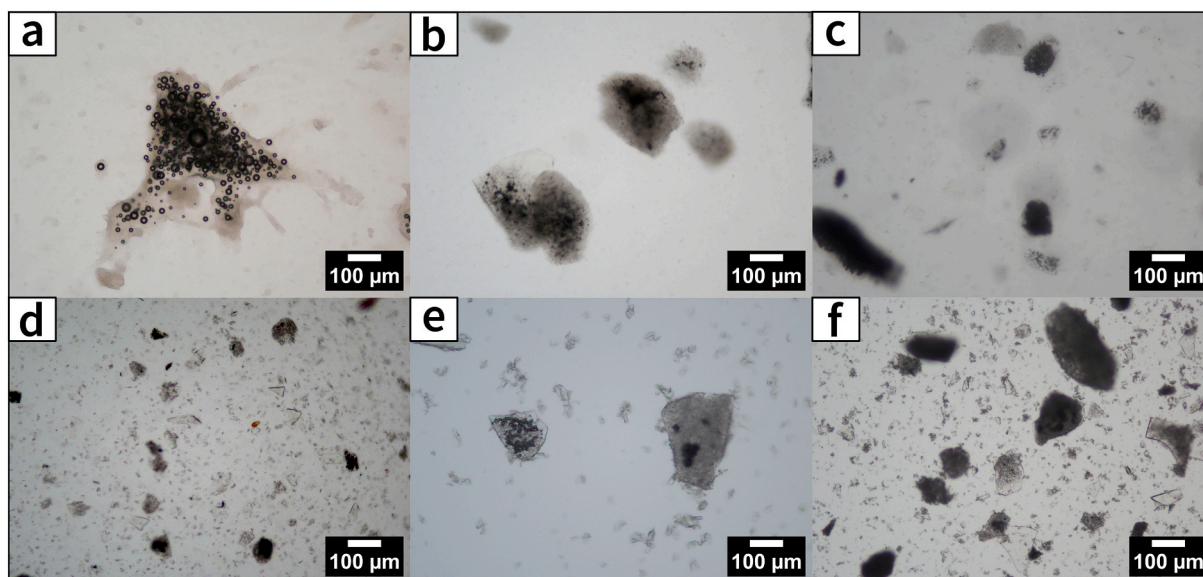


Fig. 11. Optical microscopy observations of fungal chitosan-gum Arabic dispersed in (a) deionised water, (b) glycerol, (c) sweet almond oil, (d) ethyl hexyl stearate, (e) isohexadecane and (f) ethanol.

Table 2

Active loading and encapsulation efficiency of α -tocopherol coacervates, depending on the wall-material (WM):active (A) ratio (w/w).

WM:A ratio	Yield (%)	Active loading (%)	Encapsulation efficiency (%)
1:2	73.9	6.98 \pm 0.15	77.7 \pm 1.7 ^{ab}
1:1	79.6	5.27 \pm 0.03	82.6 \pm 0.5 ^a
3:1	79.6	2.32 \pm 0.10	73.0 \pm 3.1 ^{bc}
5:1	75.6	1.54 \pm 0.02	70.0 \pm 1.0 ^c
7:1	74.9	1.25 \pm 0.03	73.5 \pm 2.0 ^{bc}

a, b, c, d values with different letters in the same column differ significantly ($p < 0.05$).

their wettability, as indicated by the lower contact angle values. Furthermore, the introduction of FC-GA coacervates in low dielectric constant solvents is a first approach in the study of the interaction of the coacervate-commonly used solvents. Isohexadecane, ethyl hexyl stearate, and ethanol can improve the wetting properties of coacervates by reducing electrostatic interactions. Conversely, polar solvents like water and glycerol may hinder the wetting properties of FC-GA coacervates due to their strong electrostatic interactions. The performance of the developed coacervates was finally assessed by encapsulating a lipophilic active: α -tocopherol with excellent encapsulation efficiency up to 82.6%. Nonetheless, given that lipophilic actives may exhibit variations in their structure and physicochemical attributes, further studies are essential for better understanding the interactions between FC-GA complex coacervates and different lipophilic molecules. The present findings undoubtedly emphasise the promising potential of fungal chitosan and FC-GA coacervates as stable materials for encapsulation suitable for high shear processes and high-temperature applications. These insights open new avenues for optimising formulations containing coacervates. Further research is needed to investigate the stability of FC-GA coacervates in different solvents and their compatibility with various active ingredients for expanding their potential applications for encapsulation in a variety of fields including pharmaceuticals or cosmetics. Given the many customisable parameters in the design of such fully natural and biocompatible and biodegradable FC/GA microcapsules, there is no doubt that the fields of application of these objects go far beyond cosmetics or food; for instance, they can readily be applied in pharmaceutical and galenic sciences. In completely different areas,

these coacervates would be promising for household products and materials where sensitive ingredients could be integrated into formulations and released when used by the consumer or over time throughout their whole lifespan.

CRediT authorship contribution statement

Adeline Delaporte: Writing – review & editing, Writing – original draft, Visualization, Resources, Methodology, Investigation, Formal analysis. **Adamantini Paraskevopoulou:** Validation, Methodology. **Michel Grisel:** Writing – review & editing, Validation, Supervision. **Ecaterina Gore:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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